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Endocrine Disruptors in PET Bottles

Introduction

Bottled water is sold in various forms including spring, alkaline, distilled, and more. An article titled “IBWA Survey Shows US Consumers Prefer Bottled Water Over Other Beverages” cited a recent online survey conducted by the Harris Poll on behalf of the International Bottled Water Association (IBWA) found that more than 63% of American respondents preferred bottled water due to it being perceived as healthy and easily available. Recent studies have called into question whether water bottled in plastic is safe for human consumption. The most popular type of plastic being scrutinized for its negative health effects is Polyethylene terephthalate (PET). This type of plastic is used by many sodas and bottled water companies around the world making it readily available for human consumption. It is well known that PET plastics have chemicals that leach into the water and are thus ingested upon consumption. In this study, we hypothesized that drinking water bottled in PET plastic has a negative effect on human health.

Polyethylene-terephthalate (PET) is a substance of ethylene glycol and terephthalic acid that's being used to produce plastic bottles. The best solution would be to minimize the production of these bottles, to just get rid of these plastics from our environment but these plastic bottles are continuously being recycled. According to Dimitrov, a member of the Croatian National Institute of Public Health, “the quantity of collected PET waste is enormously growing

but in 2007 just about 40% of all PET bottles in the market were collected for recycling.” Some of the polymers do, in fact, deteriorate during the process of recycling, but it still leaves much behind in the bottle.

Method

Many questions are arising, referring to whether or not PET bottles contain chemicals. Research shows that there are in fact chemicals in these PET bottles. To get to this conclusion, a group of scientists in Dimitrov’s study went through a specific method/process. Their goal was to analyze whether or not these PET bottles contain any chemical substances within the virgin PET bottles. Virgin, contaminated and recycled PET flakes got studied for them to keep track of any presence of contaminants, in other words, any chemicals. “PET flakes were contaminated with the test solvent mixture to simulate the worst possible scenario of contamination of bottles before recycling into new PET bottles for food application” (Dimitrov). This new product is then mixed continuously for 24 hours at a specific temperature. It then sits there for about 10 days and mixed every 2 hours. The last step from the contamination procedure is the PET flakes getting filtered, washed, and dried for 3 days before recycling by melting. Now the contaminated flakes were denoted as PET K samples. During the recycling process, these contaminated PET flakes get homogenized with thermal stabilizer Irganox and the lubricant Zn-stearate by mixing before extrusion, while the temperature is set to 220/230/230/240/240/245 degrees celsius. After, we take the virgin PET, PET K and PET RM, which is recycled PET, and analyze them.

Result

According to Dimitrov, “the pyrograms describe the peaks seen in the total ion chromatogram, which represents the number of compounds detected by GC-MS prior to thee

pyrolysis step". In this experiment, the chromatogram shows that a sample of PET contains carbon/dioxide, benzene, benzoic acid, vinyl benzoate, divinyl terephthalate, benzoic acid, Ethan-1, and diylidibenzoate. Amongst the three, PET had the most carbon dioxide at 43.93%, while PET RM has 33.61% and the virgin PET has 20.73%. But when looking at who has the most vinyloxycarbonyl, virgin PET has the most at 27.08%. Some of these elements are even impossible or extremely hard to remove. "The fact that limonene and phenyl cyclohexane are detectable in the recycled sample indicates penetration of the contaminants deeply in the PET polymer structure that is not possible to remove efficiently by cleaning and recycling process" (Dimitrov). Some of these products are also considered hazardous including benzene, phenol, biphenyl, styrene and more. It's assumed that these products are present but it's concentration increases as a result of recycling.

Method

Because antimony and Bisphenol have been known to be present in PET bottles in recent studies, Fan, Zheng, and five other researchers created a study to find out if these chemicals were safe for consumption. They investigated the effects of storage temperature and time on the release of antimony (Sb) and bisphenol A (BPA) from PET drinking water bottles in China. BPA is both a chemical and an endocrine disruptor that is often found in plastic. It's known to cause immune disorders that can affect the nervous system. According to the International Agency for Research on Cancer, antimony or Sb is categorized as a carcinogen to humans. In other words, both of these chemicals are perceived to create health risks in the human body. In this study, 16 brands of PET bottled water were bought in supermarkets of Nanjing, China. Before beginning the experiment the total amount of Sb and BPA in PET bottles had to be determined. In order to

obtain the total amount of Sb in PET plastic bottles, four bottles were taken from each brand and 0.5 g of the plastic samples were rinsed with Milli-Q-Water. After the samples were dried they were mixed with 8 mL of nitric acid and 2 ml of hydrogen peroxide and then digested on a hot plate for 4 hrs at 250°C. To determine the amount of BPA, 0.5 g of samples were soaked in ethanol for 72 hrs, used in for ultrasound-assisted extraction and then filtered with a 0.45µm nylon filter In this study to test the effect of storage temperature on Sb and BPA release from PET bottles, the three temperatures tested investigated were 4, 25, and 70°C. These three temperatures represented storage in a refrigerator, room temperature, and extremely high temperature. The water in the bottles was replaced with Milli-Q-Water and stored for seven days at 4, 25, and 70°C. After seven days the Milli-Q-Water in the bottles were sampled in order to determine Sb and BPA. To test the effect of storage duration, bottles were stored at 70°C for 1, 2, and 4 weeks. Milli-Q-water was emptied from bottles and analyzed for Sb and BPA after 1 week of storage. Then bottles were refilled with Milli-Q-Water and stored for another week to reach a total of 2 weeks in storage. After finding the amount of Sb and BPA released, the bottles were once again replaced with Milli-Q-water and put in storage and tested after an additional 2 weeks to form a total of 4 weeks in storage. When doing this experiment the bottles were not exposed to light and four replicates were used for each treatment. The concentration of Sb was determined by Inductively coupled plasma mass spectrometry (ICP-MS) and the amount of BPA was measured by High-performance liquid chromatography (HPLC).

Result

When comparing the effect of storage temperature on the release of Sb and BPA from the bottles that were stored at 4, 25, and 70°C, Fan and the other researchers noticed that the amount

of Sb increased from 4°C to 25°C in only 6 out of 16 brands. Because only six of the brands were affected, the idea that temperature from 4°C to 25 °C had an effect on the release of Sb was deemed inconclusive. However, when stored at 70°C, all brands of PET bottles showed a significant increase in Sb release levels. As for the release of BPA under different storage temperatures, researchers found that there weren't any "significant differences in BPA concentrations" until it was stored at 70°C. (Fan, p.116) When Stored at 70°C the amount of BPA released increased but not as much Sb. After measuring the effects of storage duration the researchers noticed that as the storage duration increased, the concentration of Sb increased significantly in 13 out of 16 brand PET bottles. When looking at the effect of storage duration on BPA Fan saw that it did not pose any threat to human health.

Method

In a 2008 study, Wagner & Oehlman found that consumption of mineral water bottled in PET plastics exposed humans to endocrine disruptors known as xenohormones. These hormones mimic the characteristics of estrogen and other hormones found in the body. Researchers hypothesized that the migration of substances from PET plastic bottles may expose humans to xenohormones. In this study 20 brands of mineral water (nine bottled in glass and plastic each and two bottled in Tetra Pak). The bottled water was purchased in local shops under suitable conditions for consumption (dark, 4 degrees Celcius before analysis). Three bottles of mineral water per brand were used for testing with the yeast estrogen screen (YES). The YES was used because it contains the human estrogen receptor alpha (hER α) gene. If that receptor is activated via the binding of the substrate then the enzyme β -galactosidase will be expressed. The

expression of this enzyme was measured as the change in absorbance at 540 nm when a CPRG assay is used.

To conduct this experiment researchers added 75 microliters of bottled water into a 96 well microtiter plate. The yeast culture was grown overnight to log phase. Researchers used laboratory tap water as a negative control (8 replicates per plate). 20 microliters of the yeast cells from the log-phase culture were added to each well. The microtiter plates were incubated for 24 hours using a gas-permeable membrane in order to avoid contamination. The optical density of the yeast cells was measured at 540 nm in 30-minute intervals over a 4 hour period. Optical density was corrected using a specific logistic function. In addition, a Grubb's test ($p < 0.05$) was used for more accurate results.

Result

The potency of estradiol equivalent concentrations (EEQ) was measured with the yeast estrogen screen (YES). In 12 out of the 20 mineral water brands tested the YES revealed an elevated estrogenic activity. Furthermore, researchers compared the same brand of mineral waters bottled in PET, glass, and Tetra Pak. They found a "significantly increased hormonal activity in 33% of all mineral water samples bottled in glass (three of nine brands). Compared to that, 78% of the waters from PET bottles (seven of nine brands) and both samples bottled in Tetra Pal were estrogen positive" (Wagner & Oehlmann, 2008).

Discussion/Conclusion

Our original problem was whether or not PET bottles have a negative effect on our health. In this study, we hypothesized and found that drinking water bottled in PET plastic does have a negative effect on human health. These three studies, Dimitrov, Fan, and Wagner &

Oehlmann all confirm that these PET plastics aren't healthy for the human body. During Dimitrov's study, we found that virgin PET bottles have hazardous products in them but the concentration of it is fairly small at first. After every time it gets recycled, the concentration of these products increases, making it more hazardous with time. During Fan's Study, we learn that storing PET bottles in high temperature and long duration is not recommended because of the risk it poses to health. Furthermore, in the Wagner & Oehlmann study we find that mineral water bottled in PET have an endocrine disruptor that mimics the role of estrogen. We also learned that compared to glass bottles the PET bottles resulted in double the amount of hormonal activity in the yeast estrogen screen.

Our findings prove that PET bottles do have endocrine disruptors that are known to be detrimental to human health. The endocrine disruptors contaminate water bottled in PET plastics and can be easily transmitted into our body active as functionally active estrogens. Further research on this study should include longitudinal studies of people who mostly drink water bottled in PET plastic. This would provide a breadth of knowledge on the type of ailments that can be caused or linked to the use of PET plastic. More research should also be conducted on other food products that use PET plastic such as the lining in canned beans, BPA in baby bottles, and other sources of endocrine disruptors in common products made of plastic.

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